Structure of ovarioles and oogenesis in *Panorpa liui* Hua (Mecoptera: Panorpidae)

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Abstract: The ovariole structure and oogenesis have significance in exploring the phylogenetic relationships in insects. Detailed investigation on ovariole structure and oogenesis of Panorpidae could provide the evidence of phylogenetic position of Mecoptera in Holometabola. The ovariole structure and oogenesis of the scorpionfly *Panorpa liui* Hua were investigated using light microscopy and scanning and transmission electron microscopy. The results showed that each of the two ovaries consists of 12 polytrophic ovarioles, which can be differentiated into the terminal filament, a germarium, a vitellarium and a pedicel. Based on the variations of the nurse cells, oocyte, and follicle cells, the process of oogenesis can be divided into five stages: early, middle and late previtellogenesis, vitellogenesis and choriogenesis. During the vitellogenesis, the nurse cells provided the nutrition for the oocyte and then degenerated gradually. When the nurse cells disappear completely, the nutrition of the oocyte might be supported by the hemolymph through the interspace between follicle cells. Three types of follicle cells surrounding the oocyte are responsible for the eggshell formation in different regions, resulting in the distinct meshwork on the eggshell. Through comparison with the nurse cell number in other orders, we speculate that three nurse cells in each egg chamber might be a plesiomorphic character, suggesting that Mecoptera represent a basal lineage in Holometabola.

Key words: Mecoptera; Panorpidae; ovary; histology; ultrastructure

The fundamental components of the reproductive organ in female insects are paired ovaries, each of which consists of various ovarioles in which oogenesis occurs (Gullan and Cranston, 2005). According to the ultimate fate of the developing germ cells, two basic morpho-functional categories of insect ovarioles are traditionally recognized: panoistic and meroistic (Chapman, 1998). Meroistic ovarioles can be further subdivided into polytrophic and telotrophic types based on the manner in which the oocyte is nourished (Büning, 1994; Biliński and Büning, 1998). Comparative studies indicate that oogenesis and ovariole structure can be used in phylogenetic considerations and species confirmation (Büning, 1994, 1998; Hou and Hua, 2008).

Panorpidae, the most speciose family in Mecoptera, contain over 400 species assigned to four genera worldwide (Penny and Byers, 1979; Cai *et al.*, 2008). The general structure of the ovary has been reported in *Panorpa liui* Hua (Hou and Hua, 2007), and its eggshell structure and choriogenesis were studied by Ma and Hua (2009). Herein the structure of ovarioles and oogenesis in *P. liui* were investigated using light microscopy and scanning and transmission electron microscopy in order to provide more evidences of the phylogenetic study of the order Mecoptera in the Holometabola.

1 MATERIALS AND METHODS

Female adults of *P. liui* Hua, 1997 were collected in early August 2006 at Shenyang Botanical Garden (41°49′N, 123°34′E, elev. 52 m), Shenyang, Liaoning Province, northeastern China.

For light microscopy observation on histology, the insects were fixed in Bouin's solution (saturated picric acid : formaldehyde : glacial acetic acid = 15 : 5 : 1 v/v) for 24 h before being stored in 75% ethanol. The insects were dissected under a Nikon SMZ1500 Stereoscopic Zoom Microscope. For transmission electron microscopy (TEM) observation, the materials were fixed in 2.5% glutaraldehyde in phosphate-buffered saline (PBS, 0.1 mol/L, pH 7.2) for 12 h at 4℃. They were rinsed in the same buffer and post-fixed in 1% osmium tetroxide for 2 h. After dehydration in a graded series of acetone and infiltration in series

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mixture of Epon 812 and acetone, the samples were cut into different segments and embedded in pure Epon 812. The semithin sections were stained with 0.5% toluidine blue, examined under an Olympus BX-51 microscope and photographs were taken with a digital camera. Ultrathin sections were coated on copper grids and doubly stained with uranyl acetate and lead citrate, examined under a JEOL JEM-1230 transmission electron microscope at 80 kV.

For scanning electron microscopy (SEM) observation, the dissected eggs from the lateral and common oviducts were fixed in 2.5% glutaraldehyde in PBS for 12 h at 4℃ and dehydrated in a graded ethanol series. After dried in a critical point-drier, the materials were coated with gold in a sputter coater, mounted with double-sided adhesive tape to stubs, and examined under a JEOL JSM-6360 LV scanning electron microscope at 15 kV.

2 RESULTS

2.1 Gross morphology of the ovarioles

The ovaries of adult *P. liui* are composed of 12 polytrophic ovarioles, which are developmentally synchronized. Each ovariole has its own sheath (tunica propria), the outer sheath (tunica externa), and a common ovary envelope surrounding the entire ovary (Plate II: B). In the ovariole of mature females that have not laid eggs yet, a plug formed by numerous epithelium cells stuffs the posterior end of the ovariole to prevent the oocyte flowing into the lateral oviduct (Plate I: C). From the top downwards, the ovariole tube diameter increases gradually and can be divided into the terminal filament, a germarium, a vitellarium, and a pedicel, linking the ovariole to the lateral oviduct (Plate I: A, B).

The germarium contains germ cell clusters and somatic prefollicular cells densely arranged (Plate I: B). The differentiation of the cluster has already occurred. Three nurse cells plus one oocyte differentiated from the cluster are hardly to be distinguished since each of them bears a similar volume and each cell bears a rounded nucleus which nearly takes up all the volume of the cell (Plate I: B; Plate II: A). In the posterior germarium, differentiating germ cell clusters are separated by somatic prefollicular cells. The intercellular bridge rims were observed to be embedded in fusomal and polyfusomal material at the beginning of the cystocyte differentiation (Plate II: A). The vitellarium takes up the most part of the ovariole in length and comprises 10 – 12 visible egg chambers which increase gradually in volume (Plate I: A). Each visible egg chamber is built of an oocyte and three nurse cells, surrounded by a layer of follicular epithelium (Plate I: C, E, F).

Based on variations of the nurse cell, oocyte, and follicle cell, including their volumes, contents, and especially the yolk protein in the oocyte, the oogenesis process of *Panorpa* can be divided into five phases: early, middle and late previtellogenic, vitellogenic, and choriogenic (postvitellogenic) stages (Plate I: A, D).

2.2 Early previtellogenesis

In early previtellogenic stage oocytes are localized in the apical part of ovariole (Plate I: A). The flattened and triangular oocytes grow slowly with their nuclei indistinct, while the nurse cell grows tremendously with large nuclei (Plate I: A). Nuage material accumulates in perinuclear region of the nurse cell (Plate II: B). The flattened follicle cells are sparsely located around the egg chamber (Plate I: B).

2.3 Middle previtellogenesis

Middle previtellogenic oocytes grow much faster (Plate I: A). The oocyte nucleus (germinal vesicle) located in the center is relatively large and roughly spherical, and contains a prominent nucleolus (Plate I: A). Flattened follicle cells with large nucleus keep on growing and multiplicating to surround the egg chamber, especially the oocyte, densely (Plate I: A).

2.4 Late previtellogenesis

During the late previtellogenesis, oocytes become drum-shaped, with the germinal vesicles translocating to the peripheral ooplasm and considerably increasing in volume with prominent nucleoli (Plate I: E). The nurse cells nearly reach their maximal volume, containing lots of nutrimental material. Their nuclei almost occupy the whole cell with an irregular nuclear membrane (Plate I: A). The follicle cells in this stage show four distinct subpopulations distinctly: the stretched follicle cells (FC1) covering the anterior end and the lateral sides of nurse cells, the centripetal follicle cells (FC2) migrating between the oocyte and nurse cells, the columnar main body follicle cells (FC3) surrounding the lateral sides of the oocyte, and the posterior follicle cells (FC4) covering the posterior pole of the oocyte (Plate I: E, F). In the TEM, the follicle cells of the second type were observed to increase by mitosis division and these new cells wedge in the centripetal follicle cells and attempt to enclose the oocyte densely (Plate II: C).

2.5 Vitellogenesis

In the vitellogenic stage, the roughly spherical

oocyte becomes much larger than the associated nurse cells, encompassed by mono-layered cylindrical follicular cells (Plate I: C, F). At the beginning, some small yolk granules are present at the brim of oocyte (Plate I: C, F). With the small yolk granules congregated, the nurse cells are degenerated gradually (Plate I: C, F). When the nurse cells disappear completely, a distinct interspace between follicle cells occurs and numerous mitochondria are present in the follicle cells (Plate II: D). The cell membrane of the oocyte projects numerous microvilli facing the follicle cells and dense mitochondria exist in the periplasm of the oocyte (Plate II: D). The spherical germinal vesicles remain at a characteristic lateral position in the oocyte (Plate I: C).

2.6 Choriogenesis

When follicle cells densely enclose the whole oocyte, which is fully filled with yolk granules and lipid droplets, they begin to secrete eggshell around the oocyte (Plate I: D). Firstly, a continuous layer of vitelline membrane is formed (Plate I: D). Subsequently, the follicle cells begin to secrete chorion, which has ridge processes in cross-section (Plate I: D). Under the SEM, it is worth noting that the patterns of the anterior and posterior poles are different from other regions. The impression is radial on the two poles and network on the other regions (Plate II: E, F).

3 DISCUSSION

The ovary structure and oogenesis have been widely studied in different insect groups (Büning, 1994, 1998). In Mecoptera, the females of the most families possess polytrophic ovarioles (Simiczyjew, 1996, 2002; Biliński and Büning, 1998; Biliński *et al.*, 1998). The ovarioles of Boreidae and Nannochoristidae, however, are of secondary panoistic type (Biliński and Büning, 1998). On the basis of the ovary types in the entognathans, the panoism is the primitive type and the secondary panoism (neopanosim) is evolved from the polytrophic type (Büning, 1994). According to this proposal, the Panorpidae and other groups which possess the polytrophic ovarioles should be more primitive in Mecoptera. However, this argument conflicts with the phylogeny established on the morphology and molecular evidences (Kaltenbach, 1978; Willmann, 1987; Whiting, 2002).

Based on variations of nurse cells, oocyte, and follicle cells, the oogenesis process of *Panorpa* is firstly divided into five stages: early, mid and late previtellogenesis, vitellogenesis, and choriogenesis. During the early oogensis stage, the nurse cells are mainly responsible for nutrition support for the oocyte (Büning, 1994, 1998). When the nurse cells are degenerated, the nutrition transferred to the oocyte might be supported by the hemolymph through the interspace between follicle cells. The follicle cells might take part in the synthesis of the nutrition acquired through numerous microvilli on the cell membrane of the oocyte (Plate II: D). According to the morphology of the eggshell, we suggest that different types of follicle cells participate in choriogenesis at different regions. The centripetal and posterior follicle cells secrete the chorion at the anterior and posterior poles, respectively, forming the peculiar radial impression, with several micropyles at the anterior pole (Ma and Hua, 2009; Ma *et al.*, 2009).

Generally the cytoblast in polytrophic ovaries undergoes a fixed number of division cycles for a given species, eventually forming a cluster of nurse cells and an oocyte (Büning, 1994; Matova and Cooley, 2001). The number and process of mitotic division are not universal for all insects, resulting in various nurse cell numbers in different insects. In dermapterans there is only one nurse cell accompanying an oocyte (Büning, 1994; Tworzydlo and Biliński, 2008). In most species of Phthiraptera, each follicle is composed of an oocyte and seven nurse cells (Zelazowska and Biliński, 2001; Zelazowska and Jaglarz, 2004). In the fruit fly *Drosophila* and adephagan beetle *Dytiscus marginalis*, the cystoblast undergoes four mitoses, producing 15 nurse cells and one oocyte (Koch and King, 1966; Koch *et al.*, 1967; Büning, 1994; De Cuevas and Spradling, 1998). In strepsipterans seven cycles of mitotic division produce about 130 cells in a cluster (Büning, 1994). In Hymenoptera the nurse cell number varies from 15 in *Solenius vagus* to about 50 – 60 in *Athalia rosae ruficornis* and *Apis* (Büning, 1994). According to the synopsis of the ovariole types among insect orders by Büning (1994), in meroistic ovary insects the order Dermaptera is located at the basal branch, while the order Diptera at the furthest branch. Based on comparison of their nurse cell numbers, we postulate that the number of the nurse cell in an egg chamber might reflect their evolutionary degree and the advanced groups might have more nurse cells accompanying the oocyte through more division cycles. Three nurse cells produced by only two cycles of mitotic division in *Panorpa* should be the minimal number in Holometabola, implying that

Panorpidae might represent a relatively basal lineage in Holometabola.

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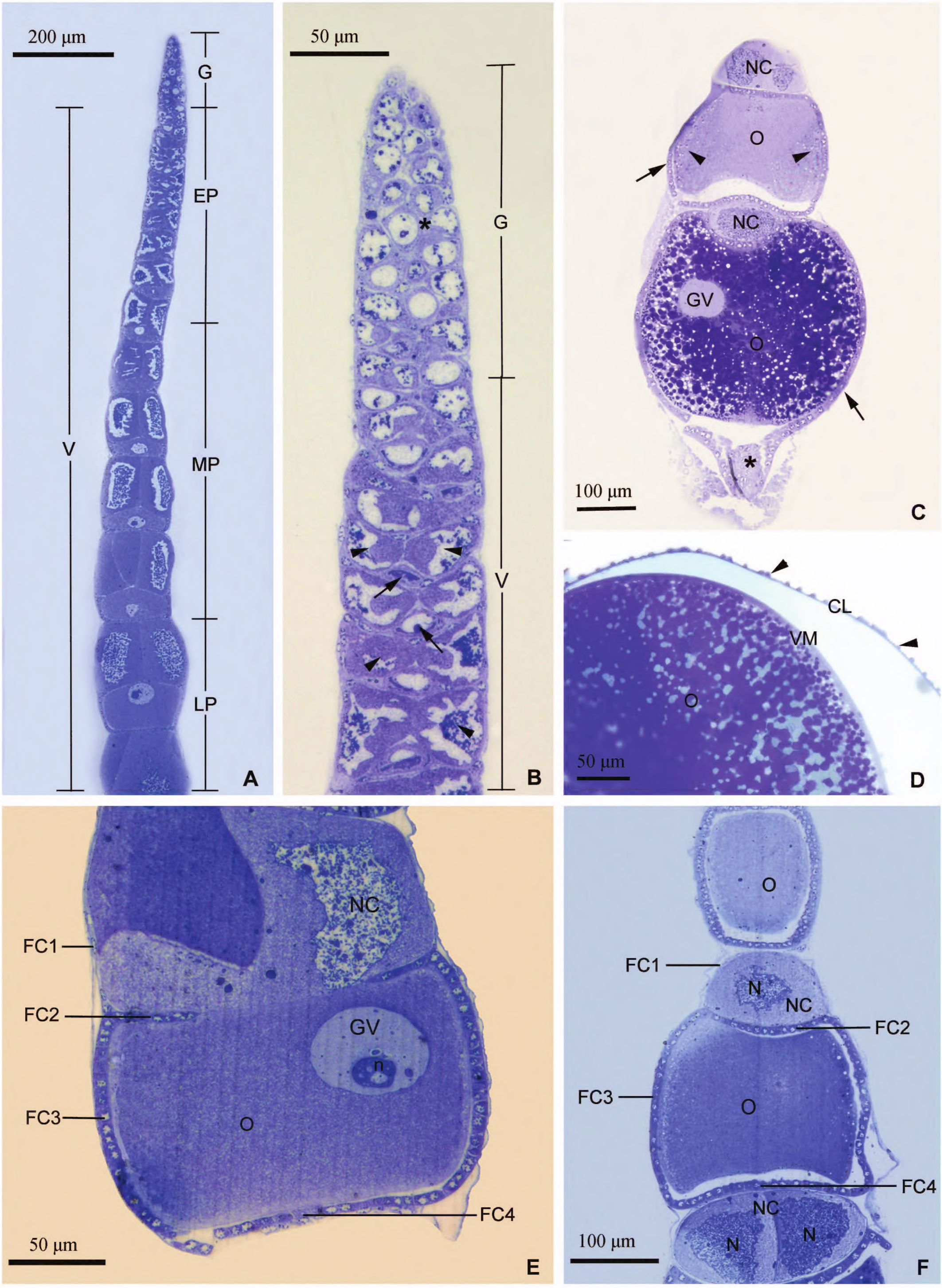
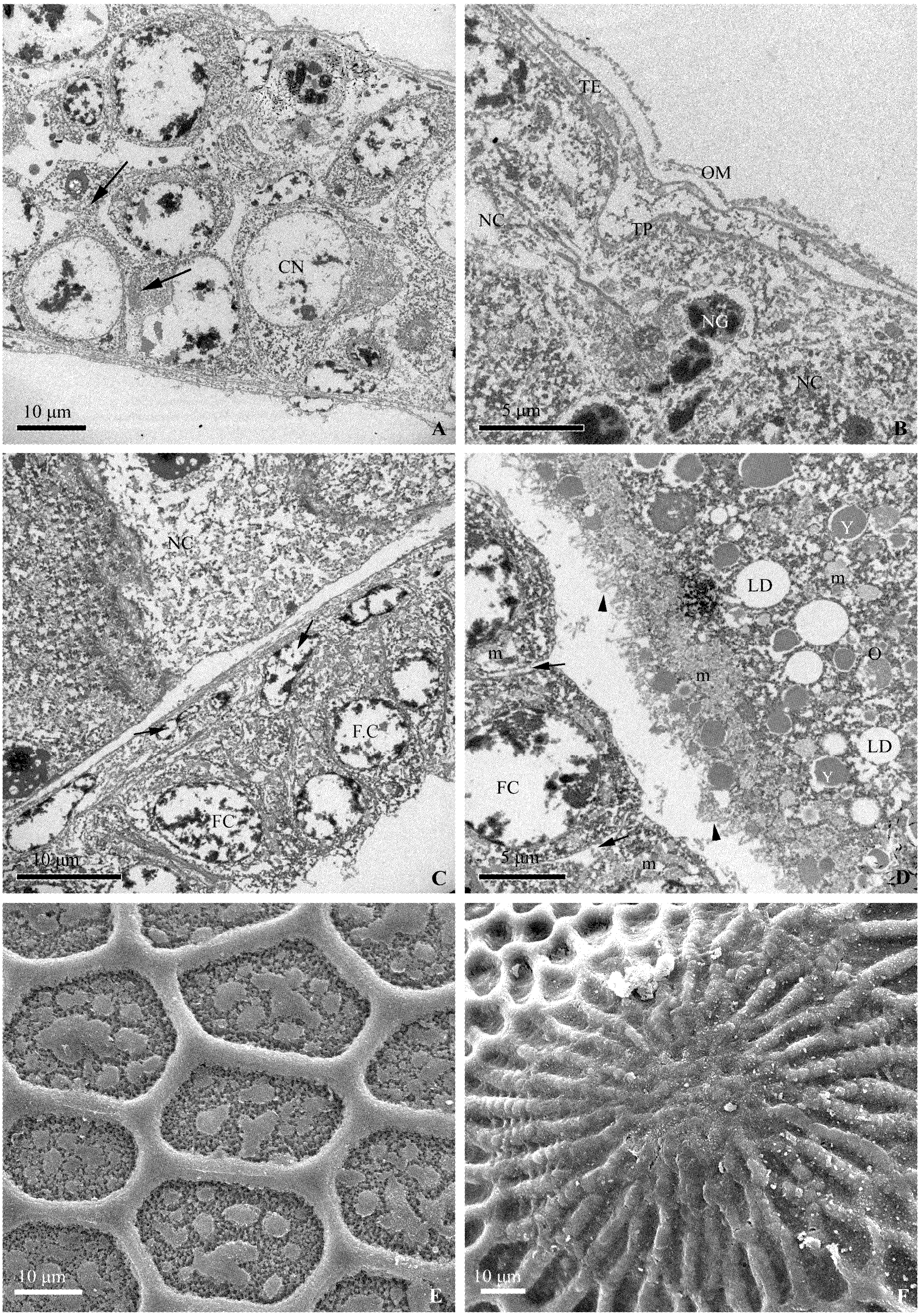


A, B: Longitudinal section of the ovariole, showing the stages of the oogenesis. Note the cystocytes (asterisk) in the germarium, nurse cells (arrowheads) and oocyte (arrows). C: Egg chambers at the base of ovariole, showing the plug (asterisk) at pedicel part. Arrows and arrowheads show the follicle cells and yolk granules, respectively. D: Cross-section of the mature oocyte at the posterior end of the ovariole. Arrowheads show the ridges of the eggshell. E, F: Subpopulations of follicle cells surrounding the egg chamber. O: Oocyte; CL: Chorion layers; EP: Early previtellogenesis; FC1: Stretched follicle cells; FC2: Centripetal follicle cells; FC3: Columnar main body follicle cells; FC4: Posterior follicle cells; G: Germarium; GV: Germinal vesicle; LP: Late previtellogenesis; MP: Mid-previtellogenesis; N: Nucleus of nurse cell; NC: Nurse cell; V: Vitellarium; VM: Vitelline membrane.



A: TEM of the cells in germarium. Note the cystocyte nucleus and prominent fusomes (arrows). B: Layers of sheaths surrounding the ovariole. C: Follicle cells (arrows) undergoing mitotic division between anterior pole of oocyte and nurse cells. D: Follicle cells and oocyte in vitellogenic stage. Arrowheads and arrows show the microvilli located on the cell membrane of the oocyte and the interspaces between follicle cells, respectively. E, F: SEM of the egg dissected from the ovariole end, showing the eggshell in the lateral region and the anterior pole, respectively. CN: Cystocyte nucleus; FC: Follicle cell; LD: Lipid droplets; m: Mitochondria; NC: Nurse cell; NG: Nuage material; O: Oocyte; OM: Ovary membrane; TE: Tunica externa; TP: Tunica propria; Y: Yolk granules.

刘氏蝎蛉卵巢管结构和卵子发生

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摘要：卵巢管结构及卵子发生过程在探讨昆虫系统发育关系中有重要意义，深入研究长翅目昆虫卵巢管结构及卵子发生可为确定其在全变态类昆虫中的系统发育地位提供依据。本文利用光学显微镜和扫描、透射电子显微镜技术研究了刘氏蝎蛉 *Panorpa liui* Hua 卵巢管超微结构及卵子发生过程。结果表明：蝎蛉卵巢由 12 根多滋式卵巢小管组成，每个卵巢小管分为端丝、生殖区和生长区。根据滋养细胞、卵母细胞及滤泡细胞的变化，卵子发生过程可分为 5 个阶段：卵黄发生前早期、卵黄发生前中期、卵黄发生前后期、卵黄发生期及卵壳形成期。在卵黄发生期，滋养细胞为卵母细胞提供养分后逐渐消亡，而此时的卵母细胞可通过滤泡之间的细胞间隙从血淋巴中获取营养。在卵壳形成期间，3 种不同类型的滤泡细胞参与形成不同区域的卵壳，从而形成不同花饰的卵壳表面。据此推测，与其他目的滋养细胞数目相比，每个卵室中 2 次有丝分裂形成 3 个滋养细胞可能是比较原始的特征，表明长翅目昆虫可能是全变态类群中近基部的分支。

关键词：长翅目；蝎蛉科；卵巢；组织学；超微结构

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